indicates some uncertainty on the subject, and my own experience of the species has now led me to conclude that what has been described as the poor- or power-cod (*Gadus minutus*) by several authors is only the young of the bib. Considerable change occurs in the outline of the fins as the adult condition is reached, and the pigment is also increased; but a large series from various parts of the British seas leaves little doubt as to the identity of the two forms.

It would appear that the confusion in regard to this species has partly arisen from an examination of preserved specimens. This is probably one of the reasons why they are separated in Dr. Günther's valuable and laborious Catalogue of the Fishes in the British Museum*.

It is remarkable that very few males were procured last season, and this out of a large number of examples obtained for examination at the marine laboratory.

XLII.—*Early Stages in the Development of the Food-Fishes.* By Edward E. Prince, St. Andrews Marine Laboratory†.

During the spring and summer of last year (1885) the ova of about twenty species of shore and deep-sea Teleosteans were studied in the Marine Laboratory, St. Andrews. Of these about half were carried through the embryonic stages in the tanks of the laboratory, and several species have, for the first time, been studied and the embryos reared at St. Andrews. Six of the species referred to have claimed special attention on account of their economic importance, and the following observations refer mainly to these, viz.:—*Gadus merlangus*, *Gadus æglefinus*, *Gadus morrhua*, *Trigla gurnardus*, *Pleuronectes flesus*, and *Pleuronectes limanda*.

So far as investigations at present show, this remarkable fact has been established—that, with the notable exception of the herring, the ova of those marine fishes which are of chief commercial value are pelagic, and when mature present almost identical features in structure and appearance. In the course of development likewise few points of dissimilarity appear; but the warning expressed by Prof. Ray Lankester is none the less just, that each form should be investigated in detail, for "in embryology the practical lesson is daily being more

* Vol. iv. pp. 335, 336 (1862).

† Communicated by the Author, having been read at the Aberdeen Meeting of the British Association (Section D), September 1885.

and more impressed upon naturalists, that the assertion of generality throughout a class or phylum of organisms for a phenomenon observed only in two or three, or even more members of that class, is an exceedingly risky proceeding"*. Each species has been studied separately and continuously in the laboratory, and much material has been accumulated, which has yet to be exhaustively worked out, and the present paper is chiefly of the nature of a preliminary account.

Spermatozoa.

The spermatozoa of the different species present no special features and are not readily distinguished from each other. They exhibit, as usual, an enlarged portion or head, which is almost perfectly spherical, with a smooth, refractive, cortical portion and a central translucent part. The vibratile filament, or "tail," is very long, delicate, and homogeneous. They issue from the fully developed male as a whitish fluid, of a rich creamy consistency, and they become diffused very rapidly in sea-water. A large quantity of milt is produced, and it is often expelled with great force. The vitality of the spermatozoa is considerable; a small quantity exposed upon a slide for three hours exhibited active movements at the end of that time.

The Ovarian Ovum.

When approaching maturity the ovum gradually loses the opacity which characterizes it for some time after protrusion from the ovarian stroma. The capsule is disproportionately thick and very pliant; but it is structureless and destitute of the radiating canals or striæ seen in many ova. Minute spherules and refrangible particles are abundant in the fluid contents, and the *vesicula germinativa* is comparatively large and usually shows a very distinct nucleolus. The intra-ovarian eggs do not ripen simultaneously, and in *T. gurnardus* especially few ova appear to mature at the same time, so that the spawning-process would appear to be protracted and intermittent, less so in the Gadidæ and still less prolonged in the Pleuronectidæ.

The Mature Ovum.

The mature ovum is more or less spherical, and when healthy has an almost crystalline transparency. It exhibits (1) a deutoplasmic globe, chiefly food-yolk, homogeneous and colourless, and destitute of large oil-globules, save in the case of *T. gurnardus*, which possesses a single spherical oil-

* Quart. Journ. Micr. Sci. vol. xvi. p. 377.

globule of a pale salmon-tint; (2) a delicate cortical film of protoplasm, in which small vesicles and granules occur; (3) a narrow space, the "breathing-chamber" of Newport*, separating the vitellus from the external capsule, and permitting it to revolve freely within the latter; lastly, the ovum possesses (4) an external protective membrane, the yolk-sac of Ransom †. It is structureless, tough, hyaline, destitute of pores or striations, slightly resilient, and varies in thickness in different species, though always comparatively thin and of great transparency. It is of uniform thickness in the same ovum, being most tenuous in *P. limanda,* measuring not more than ·0001 inch in thickness. It is slightly denser in *P. flesus*, ·000127 inch; in *Gadus morrhua* it measures ·00025; but it is considerably thicker in the ovum of *T. gurnardus*, being no less than ·0005 inch. One aperture pierces the capsule, and its structure is the same in the several species, exhibiting an "hour-glass" form, with a crater-like external and a larger internal opening.

Deposition.

As the ova mature they pass posteriorly, and descending to the genital aperture are readily expelled. Differences in the manner and duration of spawning doubtless obtain, as already stated, in the various species of food-fishes; but the ovarian walls, assisted by the abdominal parietes, at this time much distended, probably in all cases effect the extrusion of the ova. Very slight pressure upon the abdomen of a well-developed female causes the eggs to issue in a continuous stream, and artificial spawning may be easily performed. Amongst the Pleuronectidæ cases frequently occur of egg-bound females, in which the contained ova are translucent and mature, but, from difficulty in expulsion, are retained beyond the proper time. Such ova, when artificially extruded and fertilized, may develop in due course, though others under observation did not survive. A lubricating fluid facilitates expulsion; but it possesses little adhesive property, and is not hardened by contact with sea-water, unlike demersal or non-pelagic ova, which are often firmly bound together by this means. It is an interesting fact that the undetermined ova studied by Häckel and E. van Beneden ‡, though pelagic, adhered together in masses, and that the ova of *Lophius piscatorius* float in masses of mucus. Upon expulsion the buoyancy of these pelagic ova is at once apparent. Though unfertilized they rise to the surface, as Prof. M'Intosh and

* Phil. Trans. vol. cxli. 1851. † Ibid. vol. clvii. p. 433.
‡ Quart. Journ. Micr. Sci. vol. xviii. 1878, p. 42.

Dr. Hensen noted *, and congregate in scattered groups like oleaginous globules, but show no tendency to adhere together. They float freely and are carried about by the slightest current in the surrounding water. In still water they often congregate in masses and form layers, the uppermost stratum being pushed to the surface by the buoyancy of those underneath. Usually, however, they occur sparsely scattered over large spaces, and in the open sea, except in certain areas, they are so widely dispersed as to be rarely procured. Their buoyancy Dr. Wallem considers to be favourable to their development; and, in reference to the cod, when the eggs, from the more exposed Norwegian spawning-grounds, are (he says †) "carried away by currents to a calm and secure place on the lee-side they will be hatched under favourable circumstances, and the fry will find an abundance of hiding-places and food along the coast."

Fertilization.

Pelagic ova float near the surface of the water for some hours; but their buoyancy is affected by various conditions, especially adulteration of the sea-water surrounding them ‡. If no spermatozoa come into contact with them, in from two to eight hours, their translucency becomes impaired, and decending to the bottom they assume a milky opacity, the capsule becoming wrinkled and distorted. This is probably the fate of vast numbers of pelagic ova in our seas; and Hensen §, indeed, found in the inner bay of Kiel quantities of non-living ova of plaice and cod while dredging in 1881. Unfertilized eggs of *T. gurnardus* occasionally assume a bright pink colour, the cause of which has not been satisfactorily determined. The minute vesicles and granules suspended in the protoplasmic investment of the yolk persist for thirty or forty minutes after fecundation and then slowly become less abundant. The entrance of the spermatozoon was never actually seen, although successive series of ova were prepared and carefully watched in the laboratory, and active spermatozoa were seen clinging to the external capsule; but it is probable that each ovum admits only one spermatozoon through the micropyle. The fertilized ovum is readily distinguished from those in an unimpregnated condition by its more trans-

* U. S. Fish. Comm. Report, 1882, p. 434; see also Report of Royal Comm. on Trawling, 1884, p. 36.

† Int. Fish. Exh. Lond. Conference Papers: 'Fish Supply of Norway,' F. M. Wallem, p. 9.

‡ *Vide* 'Report of H.M. Trawling Commission, 1884,' p. 362, and 'Second Annual Report of Fishery Board for Scotland,' App. F, p. 47.

§ U. S. Fish. Comm. Rep. 1882, p. 434.

lucent and tense appearance. Several large enucleate cells often occur near the centre of the yolk at this early stage, but their significance and fate are not known.

Formation of the Blastodisc.

At the lower pole of the yolk-globe the film of pale ochre-tinted protoplasm increases in thickness, and the entire surface of the vitellus appears corrugated. These ridges are, however, very faintly indicated, and they mark meridional areas of transference, along which much of the cortical protoplasm passes to the germinal pole.

Segregation of protoplasm probably continues during the whole process of cleavage ; but it is most apparent during the first hour after fertilization, when the disc is being formed, as a plano-convex cap of a faint-straw-tint, in which granules sparsely occur and one or more larger vesicles appear. The disc increases not only by peripheral, but also by subgerminal transference, as is shown by the fact that vesicles and granules may be distinguished, situated partly in the disc and partly in the underlying matrix. Viewed from above, the disc is almost perfectly circular, and has the form of an inverted *plaque* depending from the yolk, the food-yolk being thus uppermost, in contrast with the Amphibian ovum, in which the animal pole is uppermost and the large food-yolk cells occupy the lower pole. The vitellus, with its germinal pellicle, revolves freely within the capsule, and the embryonic area can thus maintain its ventral position when the ovum is turned over.

Segmentation.

A central cavity soon appears below the blastodisc, by which it is lifted away from the yolk, except at the periphery, where the continuity of the disc and the periblast is never broken. This dehiscence was noted in *T. gurnardus* while the first cleavage was in progress, and in other Teleosteans a similar cavity has been noticed at an early stage ; but it is not usually regarded as representing the true segmentation cavity, the latter being recognized only when the later multi-celled stage is reached. The first cleavage is incomplete, *i. e.* the disc is not sharply separated from the periblast, and the two blastomeres are confluent below. The second cleavage cuts the first furrow at right angles, and four blastomeres result. Asymmetry is very frequent at the first cleavage, and the two cells show great disparity in size. Doubtless the phenomenon is due chiefly to unequal transference of the diffused protoplasm of the yolk; but the form and size of the cells are altered by an inherent power of movement which the constituent protoplasm possesses. To this movement are due the creases and furrows continually diversifying the surface of the blastomeres during cleavage, as well as the retrogressive process by which blastomeres reunite occasionally after cleavage. Irregularities in segmentation are far from unfrequent*, fourteen or eighteen cells being produced, and the outline of the segmenting blastodisc is thus varied, though the circular contour is always restored when the multicelled stage is reached. The blastomeres thus do not always increase with that serial regularity of geometrical progression which typical segmentation illustrates. Cleavage in a plane parallel to the upper surface of the disc commences when the blastoderm is multicelled, *i. e.* consists of from fifty to eighty blastomeres, and the form of each cell is altered by the increased pressure of adjacent cells ; the original rounded or amorphous outline being lost it becomes polygonal. The constituent cells simply consist of naked protoplasm, and at the same stage vary very much in dimensions, exhibiting a large, clear, more or less central nucleus, which is not, however, always distinguishable ; indeed there is evidence to show that periods when the nuclei of the blastoderm and periblast are visible alternate with periods when the nuclei are diaphanous. A similar rhythmical alternation may be observed in the process of cleavage, a discontinuity marked by alternations of activity and quiescence.

Segmentation is not confined to the limits of the disc, but at the margin the protoplasm of the periblast forms elevations between which the lines of cleavage extend, and cells are thus outlined in the investment of the yolk, which without doubt must be added to the blastodermic mass.

The Periblast.

Cells formed, as just described, beyond the margin of the disc contribute to the increase of the embryonic area ; but such periblastic additions to the blastoderm appear to be very limited, and it is rather by an imperceptible process of intussusception that its increase must be accomplished and the decrease of the vitellus accounted for. The lines of cleavage cannot be traced far over the surface of the periblast ; they are most distinct in proximity to the periphery of the disc, and more remotely they pass insensibly away. The nuclei of the periblast, which are more or less oval and well defined

* At St. Andrews irregularity was most frequent in the ova of *T. gurnardus* ; but Ryder noted the same feature in the cod (U. S. Fish. Comm. Rep. 1882, pp. 486–7, pl. ii. fig. 12).

and possess a nucleolus, appear at first close to the margin of the disc and are crowded together, but soon are distributed over a wide though variable area called the "nuclear zone." The origin of these nuclei is still undecided, and as they appear primarily quite at the periphery of the blastoderm, and increase row by row over the periblast-stratum, they have been derived by some authors from the nuclei of the disc. Not only do they extend outwards, but, as Agassiz and Whitman noted, they extend inward beneath the disc, and are prominently seen studding the floor of the segmentation cavity. They are often more numerous in some parts of the periblast, and less numerous or wholly absent in others.

Invagination of the Rim.

Towards the close of the first or on the second day the blastodermic rim appears. Its mode of origin is uncertain, though appearances in the living ovum strongly suggest its growth as a true invaginated layer, separated from the cells of the disc above by a distinct fissure which cannot be traced to the periphery.

Henneguy * holds that the rim is really inflected, but that the outermost or "corneous epiblast" layer takes no part in the process, an opinion which is directly opposed by Kingsley and Conn. Certainly Œllacher's view (with which Ryder agrees), that the hypoblast arises *in situ* by a simple differentiation of cells, presents this formidable difficulty, that a great part of the floor of the segmentation cavity is permanently periblastic, and that the rim merely interposes between the disc and the periblast beneath the embryonic radius and in proximity to the margin. Further, the rim clearly proceeds from the periphery towards the centre, beneath the disc, and this is inexplicable if the process be one of delamination. Nor do appearances strongly favour the theory that the rim is solely derived from the periblast; but as periblast cells are undoubtedly added to the periphery of the disc, the rim is probably a derivative from both.

In *Petromyzon* the epiblast layer extends by marginal addition, by the conversion, in fact, of the non-embryonic yolk-cells into epiblast cells †; and by a like process, doubtless, epiblast and lower-layer cells in Teleosteans increase at the margin, the converted periblast cells being immediately reflected, along with archiblast cells, to contribute to the growing and extending blastoderm. With the invagination of the

* Bull. Soc. Philom. de Paris, Apr. 1880.

† A. E. Shipley, "Mesoblast of Lamprey," &c., Proc. Roy. Soc., Nov. 1885.

rim, whose growing (inner) margin is, at first, parallel to the circumference of the disc, the latter by epibolic extension thins out, and presents in optical section a crescentiform outline. At one point, however, by a proliferation of epiblast cells, a thickening is produced coincident with one of the radii of the disc.

Formation of the Embryo.

The thickened portion of the rim, just mentioned, shows from the first a central enlargement, indicating the future head of the embryo, and an alar expansion upon each side produces a broad scutiform outline. The apex of this scutum becomes the permanent cephalic extremity, and prominently projects as a protruding carina upon the sub-blastodermic matrix, whilst posteriorly the tapering trunk of the embryo is gradually defined. The greater part of this embryonic thickening is made up of epiblast cells, which constitute the axial (neural) cord. This cord grows downward and divides the undifferentiated "lower layer" cells into two lateral cuneate masses, out of which the muscle-plates are built. The dorsum of the embryo is superficially rounded and projecting, showing no trace whatever of a longitudinal medullary groove, and there is no ingrowth of the corneous layer, such as Calberla describes in *Syngnathus*, the neurochord arising as a solid rod in which for some time no neural canal develops. It is interesting to note that *Petromyzon* precisely agrees with the Teleosteans in this feature, and, in both, the medullary canal arises as a fissure, which appears at first in the thickened anterior portion and extends posteriorly, the process being simply one of dehiscence, the central cells separating to form a longitudinal vertical fissure *. The epidermic layer now separates from the neurochord, and the hypoblast becomes thickened along the ventral median line and presses upward against the ridge of the neurochord, which is thus somewhat flattened on its under surface. The central rod of hypoblast, thus differentiated and detached, is the notochord, and posteriorly it is insensibly lost in a caudal mass of indifferent cells. At an early stage, before the notochord is completely established, its cells are in close apposition to the mesoblast cells upon each side, and the two masses can with difficulty be distinguished from each other. This difficulty of clearly distinguishing the cells of the different layers is one common to all the early stages of Teleostean development.

Meanwhile the blastoderm is proceeding epibolically to

* Shipley, "Nervous System of *Petromyzon*," Cambr. Philos. Soc. March 1886; and Scott, Quart. Journ. Micr. Sci. vol. xxi. p. 145.

invest the yolk, and on the second day usually covers more than a third of its surface. Early on the third day the equator is passed, and at the fifty-fourth or sixtieth hour the blastoderm generally envelops two thirds of the yolk. The cephalic end of the embryo remains stationary in the forms under consideration; but as the caudal extremity keeps pace with the advancing periphery of the blastoderm, its increase in length must take place in the region of the trunk. The blastopore reaches its maximum at the equator of the ovum, and when that is passed its circumference continuously decreases. The rim does not increase appreciably in density or breadth, but, on the contrary, its substance diminishes, and this must be so if, as it progresses, it contributes to the investment of the yolk. When the blastopore has so far decreased as to appear merely as a minute aperture (on the fifth or sixth day) at the posterior extremity of the embryo, the rim is recognizable only as an aggregation of cells—the cells of the coalesced margin. This remnant is probably used in the formation of the anal section of the mesenteron and other structures; but it does not appear that the caudal plate is formed directly and almost solely, as Ryder maintains, out of these cells, the tail, like the rest of the trunk, increasing in length by the addition of mesoblast somites. Still more questionable is the theory of Rauber and His, adopted by Ryder, that the hind portion of the embryonic trunk exemplifies the phenomenon of concrescence, since it is not supported by study of the living embryo, and sections reveal no trace of a median fissure or line of apposition continuous with the longitudinal vertical plane of the anterior region. Indeed the caudal plane of symmetry is at right angles to the plane of symmetry in the rest of the trunk, for the tail lies sidewise upon the yolk, and apparently develops and continues in a state of torsion until the embryo is free. Further, the solid condition of this portion of the embryo is maintained till a comparatively late stage, when the medullary canal finally penetrates it, as the first median fissure which divides its cells.

The complete differentiation of the notochord coincides in many species with the closure of the blastopore. This is the case with *Gadus merlangus*, *G. morrhua*, *P. limanda*, and *P. flesus*; but in the case of *G. æglefinus* and *T. gurnardus* the closure of the blastopore is one or two days later.

Nothing noteworthy was observed respecting Kupfer's vesicle; it has the same structure in the various species, though in *T. gurnardus* (in which it was not observed until twenty-four hours or more after the blastopore had closed) it is often compound, and presents the appearance of a group of bubble-like structures enveloped by a thin protoplasmic stratum undistinguishable from the protoplasm of the underlying periblast. In some Teleosteans it can be made out very early; according to Henneguy it appears in *Salmo fario* at the time when the blastopore coincides with the equator of the vitellus; but usually its appearance immediately precedes or succeeds the closure of the blastopore.

Sense-organs, Heart, Cœloma, Wolffian Ducts, &c.

In the evolution of the sense-organs few special features can in this place be noted. The optic vesicles are always rapidly budded off when the cephalic enlargement of the neurochord is defined. They are solid and somewhat ovoid, and their cells soon show a radial disposition, as though about to dehisce along a central vertical plane in order to form a median chamber, longitudinally placed. The formation of this chamber—the cavity of the primitive vesicle—is never accomplished, and only when the ingrowth of epiblast and the formation of the concentrically laminated lens pushes the external portion inward upon the inner portion of the *bulbus oculi* is a cavity formed within the vesicle, the so-called secondary vesicle. By this involution of superficial epiblast the rim of the secondary cup is left imperfect upon its ventral margin, and this breach is the choroidal fissure. The olfactory diverticula are pushed out as modifications of the brain a little later, and on reaching the epiblast in front of the head a ganglion is formed, uniting with an epiblast thickening, from which the olfactory nerve, according to Beard *, is split off, and thus, like the cranial nerves, is partly epidermal.

Like the eye, the ear in the Teleostei originates as a solid differentiation of cells. The otocysts can be distinguished twenty or thirty hours after the blastopore has closed. They are ovate in form, and rapidly develop a lumen, which is at first a narrow fissure surrounded by dense epiblast. The lumen rapidly enlarges, the walls become thinner, and before the embryo emerges from the ovum each otocyst develops two calcareous refringent otoliths, which exhibit a marked radiate structure.

The heart is a prominent structure in the early embryo and protrudes as a solid mass of splanchnic-mesoblast cells in the centre of the pectoral region, antero-ventrally situated below the otocysts. For some time it is solid and function-

* Beard, "Branchial Sense-Organs of the Ichthyopsida," Quart Journ. Micr. Sci., Dec. 1885.

less; but the appearance of a central lumen is accompanied by faint irregular pulsations. It is a simple tubular structure, and its cellular transparent walls assume a rugous appearance. Though no hæmal fluid can be detected until some days after emergence, the cardiac contractions were first noted on the following days:—*G. morrhua*, sixth day; *G. merlangus*, eighth day; *P. limanda*, tenth day; *G. æglefinus*, eleventh day; and *T. gurnardus* on the fourteenth day. The rhythmic movements when commencing are very slow; but when they become regular they average from twenty to thirty pulsations per minute, and when the heart is in full vigour the rate increases to forty-five beats per minute, while the embryo is still within the ovum.

As early as the fourth or fifth day a primitive cœlomic cavity develops as a horizontal fissure traversing each of the lateral muscle-plates and dividing the somatopleuric from the splanchnopleuric portions. In the proximal niche of the cavity thus formed upon each side of the embryonic axis a special part is differentiated which performs the function of an excretory duct *. At a very early stage these longitudinal tubes, whose walls consist of a single layer of cubical epithelial cells, can be traced along the line where the somatopleure and splanchnopleure remain continuous, each terminating anteriorly in a crozier-shaped loop with an infundibular opening, near which is a plicated body enclosed in a capsule, apparently a single glomerulus. Shifting their position, these tubes, the Wolffian ducts, lie on the ventral side of, and pass parallel to, the *vena vertebralis*, and posteriorly they unite to form a large urinary vesicle immediately below the origin of the tail, prominently seen in the newly-emerged embryo.

Notochord and Vertebral Arches.

The notochord arises as a rod of small cells almost undistinguishable from the neurochordal and mesoblast cells; but transverse cavities soon appear, produced by the breaking up of the original cells, which again give place to more spacious chambers filled with juicy protoplasm, much vacuolated, and forming a reticulated meshwork of great complexity. Though flexible, the notochord possesses some rigidity, due to the pressure of the protoplasmic contents of the metamorphosed cells and the continuity of their membranous walls. From the outermost cells a cuticular sheath is formed, but it is thin and shows little lamination. Outside the chordal sheath a

* *Vide* Sedgwick, "Development of the Kidney," Quart. Journ. Micr. Sci. vol. xxiv. p. 64.

mesoblastic perichordal investment is developed from the innermost cells of the protovertebræ. This is the reduced representative of the thick skeletogenous tube of Selachians and Ganoids, and probably consists of a *membrana elastica interna* only, though in favourable sections of young Teleosteans two portions can be distinguished, a *membrana limitans externa*, which passes dorsally to enclose the neurochord (constituting Rathke's *membrana reuniens superior*) and sends fibres down to meet below as the *membrana reuniens inferior*. It may be doubted whether the *elastica externa*, which separates the inner from the outer half of the skeletogenous layer in more primitive fishes, exists at all in Teleosteans, though in *Mustelus*, the Rays, etc. this layer is much nearer the chorda than in the *Holocephali*, *Notidanus*, etc., in which forms it is separated by a considerable interval from the *elastica interna*. The outer layer, which we have distinguished as a *limitans externa*, may in reality be homologous with the *membrana elastica externa*, and certainly in the species here considered cartilage develops, and McMurrich observed * in *Syngnathus* a deposition of ossific matter in this external layer. As the nucleated cartilage cells arise they proceed outwards from the perichordal sheath as two superior and two inferior rami (to each developing vertebral body), forming the neural and hæmal arches respectively.

Cartilage appears to develop independently above the medulla (in the *membrana reuniens superior*), and the hæmapophyses never really meet in the middle line, just as in the cartilaginous sturgeons, but coalesce with the median dorsal cartilage, which occupies the position of the longitudinal elastic band between the distal ends of the upper rami, in *Acipenser*, for example.

Branchial Arches.

The branchial arches are indicated as ridges passing dorsoventrally in the œsophageal region some hours before hatching. They certainly remain closed for several days after their appearance, and the clefts are not open to the exterior until the cartilaginous bars are developed. These bars can be detected in course of development soon after the embryo has emerged, the first aich developing so rapidly as to distort the outline of the head. An anterior process (the maxillary) passes immediately beneath the eyes; but the hyoid arch, being nearer the middle line, is less readily made out in the

* Quart. Journ. Micr. Sci. 1883, p. 647.

living embryo, though by the fifth or sixth day after emergence it is very movable and is raised and depressed constantly, even before the oral aperture exists. Behind the second arch the four successive branchial arches are seen as stout cartilaginous rods developed in the anterior margin of each cleft. By a forward movement of the lower curved rami of these arches they become approximated, so that a transverse section, if very slightly oblique, may pass through the series with the exception of the first arch.

Fins.

At a very early stage a fold of epiblast in the post-otocystic region is the commencement of the pectoral fin. This thickened fold upon each side assumes a rude oval outline and lies in a horizontal plane upon the yolk. The proximal portion becomes narrowed and much denser, due to the median intrusion of mesoblast tissue, which pushes its way between the upper and lower epiblast cells of the fin-fold, and, ceasing before reaching the limits of the fin, gives the margin a more transparent appearance. When the embryo emerges, the fin is a stout fan-like structure and has shifted slightly from the original horizontal plane. The basal thickening still continues, and the mesoblast cells contributing to it show a tendency to assume a radial arrangement, these radial lines extending also into the thin distal border, while at the centre of the peduncle cartilage develops independently, and extends distally as a thin central plate, unconnected at the base with any pectoral arch rudiment. Whether this central cartilage develops into the *ossa basalia*, the sole remnant of the primitive fin of fishes, or breaks up into radial rods, was not made out.

Cranium.

Simultaneously the first skeletal elements of the cranium, until now a mere fibro-membranous investment of the brain, appear as two cartilaginous bars, the trabeculæ, interesting as showing at once longitudinal bifidity in the embryo. In the interspace between them the hypophysis passes down to meet the pituitary diverticulum. The roof of the oral aperture is pushed up, not only at the point where the infundibulum is formed, but along the middle line anteriorly, this median involution giving the mouth in cross section a deeply-grooved character, with a flattened base or floor. At the oral end of the notochord and along each side two dense plates of cartilage arise—the parachordals—which grow rapidly and form the thick basilar plate. This plate unites with the trabeculæ in front, and the floor of the cranium is thus completed, while the walls and roof are still membranous. The notochord, on penetrating the skull, bends down very suddenly at an angle of about 90°, and the basilar plate bends down likewise, but passes forward at a more moderate angle—this declination of the spheno-occipital plate or basilar cartilage producing a flexure of the cranial region, which is much greater than usually supposed; indeed, the floor of the skull, soon after the embryo emerges from the ovum, lies in a plane almost parallel to the plane of the branchial arches.

Stomodæum and Proctodæum.

The preceding skeletal structures are usually well developed within a week or ten days after hatching; but it is not until that time is completed (the seventh day or later) that the stomodæum is externally open. The anus is still later. Nor is this surprising, as the embryo derives all the needful nutriment from the store of yolk which protrudes so prominently on the ventral aspect of the body during these early stages. Its bulk, however, continuously diminishes, and soon after the anal aperture arises it wholly disappears. The anus in all the forms under consideration appears comparatively late.

The anal tract is thus a solid cord until the lumen of the mid-gut extends into it, the communication of the proctodæum and the anterior portion of the alimentary canal being apparently incomplete until the tenth or fourteenth day after liberation.

Hæmal System.

The vascular system cannot be treated in detail in this place; but one point demands some reference. As already stated, the heart's pulsations commence at an early embryonic stage, long before a true hæmal circulation exists. It can hardly be doubted that a colourless plasma is distributed over the trunk of the embryo, though it is impossible to detect any such lymph-circulation. At a certain late stage red corpuscles do make their appearance, though whence they are derived is a question as yet undecided. Many considerations favour Ryder's view that they are directly periblastic, and some evidence, from observations on *Alosa* *, *Salmo*, and *Gastrosteus*, seems to support it. Sections of early embryos

* In the embryos of this species Ryder affirms that the venous end of the heart opens into the persistent segmentation cavity (U. S. Fish. Comm. Rep. 1882, p. 537).

in which the subnotochordal trunks are developed show an abundance of nucleated cells, of large size and spherical form (becoming polyhedral in microscopic preparations), filling up the lumen of each vessel. Those which crowd the *vena vertebralis* are strongly held by one observer * (K. F. Wenckebach) to be the original form-elements of the blood. Precisely similar cells, rounded, colourless, and nucleated, completely fill up the lumen of the aortic trunk. If Wenckebach be right, his conclusion must be extended, and the undetached cells in the aorta must be also regarded as original blood-cells, which have not yet acquired the colour and other characteristics of blood-corpuscles. In several series of the embryos of *Gastrosteus spinachia* at the St. Andrews Marine Laboratory the passage into the heart of corpuscles, detached from the yolk-cortex or periblast, was observed on many occasions †, and it is highly probable that these are hæmal form-elements; but further observations are needed. No perivitelline circulation, such as is seen in *Gastrosteus*, *Cottus*, *Liparis*, etc., was observed in any of the advanced embryos studied, though a branchial subnotochordal, caudal, and in some cases a cœliac circulation was active. Thus minor differences doubtless obtain in the development of the blood-corpuscles in various Teleosteans.

Diagnostic Features.

(1) *Ova.*—It was pointed out on a preceding page that the ova, as well as the early embryos, of the species under consideration are remarkable for the few external points of difference which they present. Their identification is often a task of considerable difficulty, and even familiarity with the various ova does not entirely remove the uncertainty of determination. Hence the desirability of establishing reliable points of difference. Arranging the ova in the order of size, which is a distinctive character sufficiently well marked to serve for determination in the laboratory, the following features may be noted:—

Pleuronectes platessa: diameter ·065 to ·069 of an inch.—The largest ovum of the various species treated of in this paper. Form spherical, hyaline capsule denser than in the two species of *Pleuronectes* mentioned below. The embryo shows pigment at an early stage of a pale yellow tint, quite distinguishable from *P. flesus*.

* Journ. of Anat. and Physiol. vol. xix., April 1885, p. 231: Wenckebach, "Development of Blood-corpuscles" (*Perca*).

† Ann. & Mag. Nat. Hist., Dec. 1885, p. 494; and U. S. Fish. Comm. Rep. 1882, p. 543.

Trigla gurnardus: diameter ·0598 of an inch.—Spherical form almost constant, spheroidal ova being rare; capsule hyaline and dense; vitellus exhibits a large, pale salmon-tinted oil-globule. During development several large cells (multinucleate) occur in proximity to the embryo, and stellate nucleated particles of protoplasm soon after closure of the blastopore occur, distributed over the surface of the vitellus.

Gadus æglefinus: diameter ·058 in.—Ellipsoidal form frequent; capsule thin and of great translucency; no oil-globules.

Gadus morrhua: diameter ·0551 in.—Ellipsoidal form sometimes preponderates; capsule hyaline, but slightly denser than in the other Gadoids enumerated here. Shows a faint bluish translucency. No oil-globules.

Gadus merlangus: diameter ·0476 in.—Very crystalline in its translucency. During development exhibits (about the seventh day) one or more enucleate structures, elaborately stellate, usually occurring one on each side of the embryo near the mid-mesenteric region; sometimes a third, asymmetrically placed, occurs. They have the form characteristic of a "bone corpuscle." No oil-globules.

Pleuronectes flesus: diameter ·038 in.—Usually spherical, but ellipsoidal form is frequent. Capsule hyaline and exceedingly tenuous.

Pleuronectes limanda: diameter ·033 in.—Hensen compares the ova of this species with the preceding (*P. flesus*) in the following terms *:—"Those of the flounder are small; but the smallest of all (less than 1 millimetre) are those of *Platessa limanda*." Capsule hyaline and very thin. The whole ovum exhibits a delicate golden-brown tinge, which is characteristic. On the fifth day after fertilization the vitellus exhibits a remarkable reticulation, apparently due to the peculiar disposition of the protoplasmic yolk-cortex. Polyhedral spaces are enclosed by the intersecting ridges, which appear to be merely superficial and therefore unlike the reticulation which penetrates the entire yolk-mass in Elasmobranchs, forming, as Dr. Schultz discovered, a series of radial lines from the centre to the circumference. Further, according to Balfour† they exist before and after fertilization, whereas in *P. limanda* no reticulation is visible until long after fertilization.

* U. S. Fish. Comm. Rep. 1882, p. 428.

† Journ. Anat. and Physiol. vol. xix. pp. 379 and 541.

(2) *The Embryos.*—There is little doubt that the pigmentation of embryonic Teleosteans is a feature of great diagnostic value. The valuable observations of Agassiz* upon this subject are well known; but with the exception of Professor M'Intosh's contributions on the subject very little has been done. The study of an extended series of embryos alone can establish the validity of pigmentation as a means of identification; but observations at the St. Andrews Laboratory lend considerable countenance to the contention that embryonic coloration is diagnostic.

Pigment appears in *P. flesus* at the earliest stage, and is, as Prof. M'Intosh describes †, "of a peculiar pale olive-brown (brownish yellow by transmitted light)," forming distinct patches on the dorsum and tail, with intervening lines of spots. Pigment of a more distinctive yellow colour—a rich amber shade—appears in *P. limanda.* Its distribution is similar to that in *P. flesus*; but in neither species does it extend over the yolk. Large stellate black pigment spots occur in the more advanced embryos of *P. limanda*, extending over the eyes, otocystic and hepatic regions to the anus, and along the dorsum and upper margin of the caudal trunk. Crescentic yellow pigment patches appear in the caudal membrane.

G. merlangus exhibits no coloration until the eighth day after hatching, when pale yellow amorphous corpuscles appear, chiefly on the dorsal and lateral surfaces; they extend also over the yolk-surface and embryonic fin-membrane. The tint is characteristic—a pale yellow with a distinctive green tinge. In the two remaining Gadoids black pigment alone appears—in *Gadus morrhua* two days before emerging from the ovum, and in *G. æglefinus* on the eleventh day, in a series which emerged on the twentieth day. In both the spots are at first amorphous and confined to the dorsal aspect of the trunk; but they rapidly extend, especially in the ventral or mesenteric region and region of the shoulder, the pectoral fins being also radially pigmented. *T. gurnardus* is scantily pigmented on the eleventh day, the spots being of a pale sea-green hue; but two days later yellow corpuscles are plentiful, and a few are of an ochreous hue. Lastly minute black spots occur. The surface of the yolk becomes rapidly pigmented as well as the protoplasmic investment of the oil-globule. It is well known that monsters frequently occur: but these were rare in the large number of embryos reared at St. Andrews.

* Proc. Amer. Acad. Arts and Sci., June 1878, pp. 1–18.

† 'Second Annual Report of Fishery Board for Scotland,' 1884, Appendix F, p. 47.

One example (*P. limanda*) possessed two heads, one head being normal, while the other was much confused. The bifurcation occurred in the mid-region of the trunk, and it is remarkable that while the alimentary tract was bifid the notochord was not so. An abnormal example of *T. gurnardus* again was malformed in the cephalic region, only one eye being developed and situated on the ventral side of the head. The otocysts were displaced, but the trunk presented no unusual features.

Conditions of Temperature &c.

It is unnecessary to say that temperature has great effect in accelerating or retarding developmental changes. Thus, in the case of *G. morrhua*, the stage figured by Ryder as the thirtieth day was reached at St. Andrews on the twentieth or twenty-first, the acceleration being due to increase of temperature. When, however, the temperature is about 40° F., a rise or fall of three or four degrees appears merely to abbreviate or lengthen development by about ten or twelve hours. The series of ova and embryos dealt with in the preceding pages were not all reared at precisely the same temperature, but by a constant flow of water from the sea outside the laboratory the temperature is kept as low as possible, and rises very gradually as the season advances. Thus from March (early in the month) to midsummer the temperature of the water in the tanks rose from 34° or 35° F. to 49° and, occasionally, 51° F. Of scarcely less moment than temperature are the other conditions, such as chemical purity of the water and freedom from detritus, mud, &c. These conditions are secured at St. Andrews by the proximity of the laboratory to St. Andrews Bay, on the beach of which the buildings stand, while the harbour passes on the north and west sides of the laboratory. Before flowing into the tanks the water pumped from the bay is retained in a spacious supply-tank until its sediment is all deposited. This course is absolutely necessary, as contact with particles of sand, mud, or mucus in the water inevitably proves fatal. Newly-hatched embryos are such delicate organisms that very slight contact with hard substances (such as contact with the side of the tank) is hurtful, while the slightest pressure at once produces opacity in the transparent embryo, premonitory of death. On emerging the young fishes swim in reversed position, yolk upward, and for some time have little power of guiding their course. By rearing them in tanks of large capacity contact with the sides is in a great degree obviated.

In studying the development of the food-fishes this conclusion is unequivocally arrived at—that the Teleostei embryologically, as also morphologically, are a highly specialized group, and are too far removed from the primitive or protichthyoid type to yield much material for broad generalizations. Attempts in that direction can hardly in any great degree prove fruitful, and must often be misleading. Abbreviation and the intrusion of secondary, and even tertiary, modifications have been so extensive that the conclusions yielded by Teleostean embryology can never have the interest or application which Selachian development possesses. But though the Teleostei, from great specialization, reveal a striking contrast when compared with such a group as the Elasmobranchs, yet investigations into their development, in which our knowledge is so fragmentary, are of great importance from many points of view, and have, it cannot be denied, an eminently practical bearing. The imperfect state of our knowledge regarding the early history and conditions of development of our important food-fishes is happily not likely to exist much longer. That the embryology of these forms is being actively pursued by many investigators is an encouraging and promising sign. Of hardly less importance is the study of those smaller forms upon which the food-fishes are to no small extent dependent for nutriment.

The writer, in conclusion, desires to warmly acknowledge his obligations to Prof. M'Intosh, whose great experience and kind advice are so freely available to those who carry on researches in the Marine Laboratory at St. Andrews. He desires to express his obligations for the use of the scientific section of the University library, for the use of the Caldwell microtome belonging to the University, and for memoirs and accessories in the zoological laboratory at the United College in the University of St. Andrews. Finally, he is under obligations to Prof. Cleland, of Glasgow, and Dr. Hans Gadow, of Cambridge, for suggestions, of which he purposes to avail himself more fully in a later (future) paper, when the preparations, only partially dealt with in this abstract through exigencies of space and time, will be treated more completely.

XLIII.—*On the Oviposition in* Phyllomedusa Iheringii.

By Dr. H. von Ihering, Rio Grande, Brazil.

Until this year, my attempts to discover the mode of reproduction of *Phyllomedusa Iheringii* had failed. I found the frog in numbers during the breeding-season, but could detect no spawn in the water near which they congregated. I have